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Synopsis Title:

Cloning and partial characterization of a gene encoding Cellobiose Dehydrogenase from *Termitomyces clypeatus*

Introduction:

Fungi play a vital role in carbohydrate metabolism by producing an extensive set of carbohydrate-active enzymes. Many of them have extensive industrial uses. Most of these enzymes in fungi are constitutive, but sometimes they are adaptive, being synthesized only in the presence of substrates. Among fungal classes, Basidiomycetes are known to be efficient degraders of cellulose [1]. Many of these species grows on dead wood or litter. The enzyme sets and understanding about fungal diversity with respect to carbohydrate metabolism are essential for the improvement of new strains and the development of enzymatic cocktails for industrial applications.

Background:

Termitomyces is a paleotropical genus of agarics which grows in association with termites and their nests and is dependent on the organic matter brought by the insects from their feeding on trees. The fungus helps the termites to degrade the plant-derived materials (e.g., wood, dry grass, and leaf litter) on which they live. *Termitomyces clypeatus* is known as a potential producer of different enzymes in culture media (Khowala & Sengupta, 1992; Khowala, Ghosh, & Sengupta, 1992). The fungus *Termitomyces clypeatus* has been found to be a potential producer of a broad spectrum of extracellular glucosidases (cellulase, sucrase, cellobiase etc.), capable of hydrolysing the polysaccharides, e.g., hemicellulose, cellulose, and starch. *Amylase and* xylanase, which are two important carbohydrate metabolizing enzymes, were purified from the culture filtrate of mushroom *T. clypeatus* [2]. Different enzymes such as endo 1, 4-β-D-Xylanase, 1, 4-β-D-Xylosidase, α-L-arabinofuranosidase, acetyl esterase, α-amylase, amyloglucosidase, were also

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purified from the fungus [3]. *T. clypeatus* produces cellobiose dehydrogenase in cellulose medium with the highest yield among all the reported fungal species [4].

Cellobiose dehydrogenases (CDH) are extracellular fungal hemoflavoenzymes produced by many white-rot, soft- rot and brown- rot fungi. CDH are monomeric enzymes carrying two prosthetic groups, a heme and a flavin domain [5]. The sequence corresponding to heme domain is located in the N-terminus, which is followed by 16- residue long region rich in serine and threonine [5]. The flavin domain in C-terminal binds FAD non-covalently. These two regions are separated by a Thr-Ser-rich long linker region. CDH displays the properties of a typical dehydrogenase with oxidative and reductive half reaction [5]. CDHs have been shown to bind cellulose in different ways depending on species. Their involvement in many reactions has been demonstrated, e.g. the production of hydroxyl radicals by a Fenton-type reaction, which may participate in the degradation of cellulose, lignin and xylan. The Fenton mechanism of CDH has a potential application in degrading and modifying poisonous aromatic waste products by increasing their water solubility [5]. CDH can function as biosensor, e.g. CDH can be used to detect lactose in food which is very important for persons with lactose intolerance. This enzyme can be used for bleaching of pulp by reduction of quinones and similar structures in the pulp. CDH also offers an attractive tool by saccharifying raw lignocellulosic material and produces appreciable amount of gluconic acid which can be fermented to alcohol by fermenting microorganisms [1].

Objectives:

1. Search for homologous regions in the known sequences of the CDH gene from other closely related organisms of *T. clypeatus*.
2. The coding gene sequence of CDH will be constructed from mRNA.
3. Characterization of this cDNA for CDH enzyme will be carried out.

Proposed plan of work:

- **Search for homologous region of CDH from other related fungal sources**: The gene sequences of the enzyme from related fungal sources are to be collected from sequence database (NCBI) and ClustalW program will be used for alignment of sequences. Homologous region can be searched from the alignment and oligonucleotide primers will be designed.
- **Total RNA isolation from *Termitomyces clypeatus:*** Total RNA will be isolated for determining the cDNA sequence.
- **cDNA synthesis:** cDNA will be synthesized by using reverse transcriptase from the total RNA.

- **Cloning of cDNA**: The total cDNA sequence obtained will be amplified using forward and reverse primer designed previously. The cDNA will be cloned into vector and will be sequenced.
- **Characterization of cDNA**: After getting the total sequence of CDH cDNA, the gene structure, enzyme structure will be determined by using Bioinformatics tool and other relevant studies will be carried out with the gene.

References:

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